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(54) Title: MONOCLONAL ANTIBODY TO AMYLOID PEPTIDE

(57) Abstract

Disclosed is a monoclonal antibody and specific-binding fragment thereof which is specifically reactive with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted and which monoclonal antibody and specific-binding fragment thereof does not react with other peptides of human origin. Also disclosed is a hybridoma cell line capable of producing the monoclonal antibody, a reagent composition which incorporates the monoclonal antibody or specific-binding fragments thereof and an immunoassay method for their use.

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MONOCLONAL ANTIBODY TO AMYLOID PEPTIDE

BACKGROUND OF THE INVENTION

Field Of The Invention

The present invention relates to neurological disorders, and, more particularly, to monoclonal antibodies that are specific for peptides associated therewith.

Brief Description Of The Prior Art

Alzheimer's disease is characterized by three major pathological markers. They are neuritic plaques, neurofibrillary tangles and cerebrovascular amyloidosis. Glenner, et al., Biochem. Biophys. Res. Comm., 120:885 (1984) and Wisniewski, et al., Int. Symp. Dementia and Amyloid "Neuropathology", Suppl. 1:87 (1986). Alzheimer's cerebrovascular amyloid protein has been purified and a 24 amino acid residue sequence has been reported. Glenner, et al., Biochem. Biophys. Res. Comm., 122:1131 (1984). It has also been observed that the sequenced portion of the cerebrovascular amyloid peptide is part of the sequence of a precursor protein. Robakis, et al., Proc. Nat. Acad. Sci. USA, 84:4190 (1987) and Robakis, et al., The Lancet, 1:384 (1987).

Down's Syndrome is a disability characterized by the inheritance of an extra copy of chromosome 21 in each cell. Older persons afflicted with Down's Syndrome display dementia that resembles Alzheimer's disease. The cerebral tissues of these individuals exhibit the same neuropathological findings of Alzheimer's disease, i.e., amyloid-containing neuritic plaques, neurofibrillary tangles and cerebrovascular amyloidosis. The amyloid deposits of Down's Syndrome contain the same peptide

as the amyloid deposits found in the brains of Alzheimer's disease victims. See, Glenner et al., Biochem. Biophys. Res. Comm., 122:1131 (1984).

As applied to other peptides and antigens, including many associated with various diseases, monoclonal antibody products of hybridoma technology have often proven valuable in studying factors associated with these diseases and in diagnosis of the disease in clinical settings. Kim, et al., Neuroscience Research Communications, 2:121 (1988) discloses production and characterization of monoclonal antibodies specific for the synthetic cerebrovascular amyloid protein described in Glenner et al., Biochem. Biophys. Res. Comm., 122:1131 (1984), supra.

SUMMARY OF THE INVENTION

Hybridoma technology is one of the most important biological tools for the analysis of complex antigens. Using the monoclonal antibodies of the present invention, which are specific to recently acquired amino acid sequences of beta - amyloid precursor proteins, it has become possible to study the beta-amyloid precursor protein which leads to significant deposits of vascular and neuritic amyloid plaques in Alzheimer's disease and Down's syndrome. These monoclonal antibodies provide the potential for non-invasive diagnosis thereof using body fluids such as spinal fluid, serum or urine.

In one aspect, the invention provides a monoclonal antibody and antigen - binding fragment thereof which are specifically reactive with a peptide whose concentration level is elevated in individuals having Alzheimer's disease or Down's syndrome as compared to individuals of substantially the same age who are not so-afflicted and which do

not react with other peptides of human origin. The monoclonal antibodies of the invention are primarily of subclass IgG_1, while others are of a subclass selected from IgG_{2a}, IgG_{2b} and IgM. Papain - digestion produced antigen - binding fragments (e.g. Fab) have also been shown to specifically recognize amyloid plaque.

The monoclonal antibody is produced by a hybridoma formed by fusion of cells from a myeloma line, usually of mouse origin, and antibody-producing cells, also usually of mouse origin, previously immunized with a peptide whose concentration level is elevated in individuals having Alzheimer's disease or Down's syndrome as compared to individuals of substantially the same age who are not so-afflicted and which do not react with other peptides of human origin, such as one which forms a portion of a cerebrovascular amyloid plaque characteristic of Alzheimer's disease or Down's syndrome. Preferably, a mouse NSO myeloma line is used, particularly with antibody-producing cells from a mouse previously immunized with a peptide having a C-terminal sequence comprising leu - val - phe - phe - ala - glu - asp - val. Examples are monoclonal antibody SCVAP-2F9 and monoclonal antibody SCVAP-4G8. Further in this regard, the invention provides a hybridoma cell line capable of producing the monoclonal antibodies described above.

In another aspect, the invention provides a composition for quantitatively determining a peptide whose concentration level is characteristic of Alzheimer's disease or Down's syndrome in an individual. The composition comprises a monoclonal antibody or antigen - binding fragment thereof in accordance with the invention and a detectable moiety which is directly or indirectly associated

therewith. In one embodiment, the monoclonal antibody is conjugated to an enzyme and the detectable moiety comprises a chromogenic redox substrate for the enzyme. In another embodiment, the monoclonal antibody is conjugated to one partner of a specific binding pair and the other partner of the specific binding pair is conjugated to a detectable moiety or a substance capable of rendering the moiety detectable. One example of this embodiment is where the monoclonal antibody or antigen - binding fragment thereof additionally serves as an antigen of the specific binding pair. The partner for the monoclonal or fragment is an anti-immunoglobulin antibody, usually anti - IgG and usually from a species other than that of its partner, which is labeled, such as with a fluorophore like fluorescein isothiocyanate. Another example is where one partner of the specific binding pair is selected from biotin and its binding analogs and the other partner is selected from avidin and its binding analogs. Usually, the detectable moiety is a chromophore, fluorophore or luminophore and the substance capable of rendering it detectable is an energy donor or catalyst therefor.

Further in this regard, the invention provides an immunoassay method. The method comprises contacting a sample, from the individual suspected of having Alzheimer's disease or Down's syndrome, with the composition of the invention and quantitatively observing any detectable response.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph illustrating that the relative OD reading between concentrations of SCVAP in the range shown can be quantitated using the method in accordance with the invention. The

sensitivity of the method is in the range of 0.2 to 0.4 ng/100 ul of SCVAP in solution.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

Monoclonal Antibody (mAB) To Synthetic Cerebrovascular Amyloid Peptide (SCVAP) and Fragments

In the experiments reported by this example, a synthetic polypeptide, an antigenic conjugate thereof and tryptic fragments of the polypeptide were used to prepare and characterize the monoclonal antibodies of the invention.

Preparation of SCVAP and Fragments

Preparation of SCVAP and its tryptic fragments was as follows. A 24-residue synthetic cerebrovascular amyloid peptide (SCVAP) corresponding to the published sequence of the Alzheimer's cerebrovascular amyloid peptide was obtained from Peninsula Laboratories, Belmont, CA. The sequence mentioned was published in Glenner, et al., Biochem. Biophys. Res. Comm., 120:885 (1984). The preparation as received was purified by reverse phase liquid chromatography and the sequence was confirmed by amino-terminal sequencing as described in Bobin, et al., Acta Neuropathol., 74:313 (1987). The purified peptide (1 mg) was conjugated to keyhole-limpet hemocyanin (6 mg) with glutaraldehyde (3 ul) in sodium phosphate (1.2 ml, 50 mM, pH 7.5) at 4°C for 2 hours to provide the SCVAP-KLH antigen.

The purified peptide was also cleaved using trypsin into fragments containing residues 1-5, 6-16, 6-24 and 17-24 as follows. The peptide (1 mg) in 0.1 M NH_4HCO_3 (500 ul) was treated with TPCK-treated

trypsin (Cooper Biomedical, Malv rn, PA) overnight at 37°C. The peptide fragments were resolved by reverse-phase chromatography on a 4.6 x 50 mm C-8 column (J.T. Baker, Phillipsburg, NJ) eluted with a linear gradient of 0-60% acetonitrile in 0.1% trifluoroacetic acid for 40 minutes at 1 ml/minute. The fragments were identified by amino analysis and the sequences were determined to be as follows:

Fragment	Residues	Sequence
1	1-5	DAEFR
2	6-16	HDSGYQVHHQK
3	6-24	HDSGYQVHHQKLVFFAEDV
4	17-24	LVFFAEDV

Amino acids are designated by one-letter symbols in accordance with the convention described, inter alia, in Lehninger, Biochemistry (2nd Ed.), Worth Publishers, Inc., New York, pg. 72 (1975). These fragments were used, as described below, to sublocalize the epitopes of the monoclonal antibodies prepared as described herein.

Preparation of Hybrid Cell Lines

Procedures for producing antibody-secreting hybrid cell lines as described in Togashi, et al., Arch. Virol., 57:149 (1981) and modified as described in Kim, et al., J. Clin. Microbiol., 18:331 (1983) were applied as follows.

BALB/CJ female mice (Jackson Laboratories, Bar Harbor, ME) were immunized via the back foot pads with Ribi adjuvant (200 ul) (Ribi Immunochem Research, Inc., Hamilton, MT) containing SCVAP-KLH antigen (25-50 ug). At 21 days after the initial injection, these mice were immunized intraperitoneally with SCVAP-KLH antigen (25-50 ug) in Ribi adjuvant (200 ul). Four days prior to

fusion, at 6 weeks after the last immunization, the mice received intraperitoneal booster injections of SCVAP (200 ug) without adjuvant. Immune spleen cells were harvested as described in Galfre, et al., Methods Enzymol., 3:73 (1981). A mouse with the highest titer after SCVAP-KLH antigen immunization was selected for hybrid clone production.

NSO myeloma cells were obtained from Dr. Choi, Kyung Hee of Albert Einstein Medical School, New York, NY. The immune spleen cells were incubated with the NSO myeloma cells and fused at a ratio of 5:1 in the presence of polyethyleneglycol 1500 (Boehringer Mannheim, Indianapolis, IN) as the fusing agent. The cells were maintained in Iscoves modified Dulbecco minimum essential medium containing 15% fetal bovine serum, 10^{-4} M hypoxanthine, 1.6 x 10^{-5} M thymidine and 4.0 x 10^{-7} M aminopterin for 14 days. Subsequent feedings with medium containing only hypoxanthine and thymidine were performed on days 18, 22 and 25. After day 25, cells were fed with medium that did not include hypoxanthine, thymidine and aminopterin. Normal BALB/CJ mouse macrophages (6,000 cells per well in 96 wells of a microtiter plate) as a feeder layer were used for the initial hybridoma production.

Establishment of Hybrid Clones Secreting Anti-SCVAP mABs

After the initial hybridoma production, as described above, 5 x 10^4 hybridoma cells in 1.0 ml were added to each well of 96 well tissue culture plates. After 2 to 4 weeks of incubation, hybrid cell growth was detected in 300 of 420 wells. The supernatant fluid in 122 of hybrids from the 300 wells were found to contain SCVAP-specific antibody as determined by ELISA, as described below.

Hybrids that produced SCVAP specific antibody, as determined by the ELISA procedure, were cloned three times by limiting dilutions to obtain pure clones. Normal BALB/CJ mouse macrophages (6,000 cells per well in 96 wells of a microtiter plate) as a feeder layer were used here also. Fifty (50) stable cloned hybrid cell lines were established and are referred to here as purified antibodies. From these, thirty (30) well-adapted, good antibody producers were grown in tissue culture flasks and further characterized.

ELISA Screening for Anti-SCVAP mABs

Hybridoma cells were tested for the production of SCVAP antibodies by the ELISA technique as described by Kim et al., J. Clin. Microbiol., 18:331 (1983). SCVAP antigen was coated onto Falcon 96 well, 3911 Micro Test 111TM flexible assay plates by adding 50 ul of SCVAP antigen (1 ul/ml) in carbonate buffer (ph 9.6) and incubating overnight at 4°C. The substrate used was 3,3',5,5'-tetramethylbenzidine (TMB) (1 ml of 10% stock solution of TMB dissolved in dimethyl sulfoxide, 100 ml of 0.1 M sodium acetate/citric acid buffer, pH 6.0, 148 ul of 30% hydrogen peroxide). Substrate solution was added to each well (100 ul/well) and incubated at room temperature for 30 minutes. The reaction was stopped by adding 2 M sulfuric acid (25 ul) to each well. The color change was measured with a Dynatech Micro Elisa Auto Reader MR580, using a 450 nm filter.

The antibody titers as determined by ELISA for tissue culture supernatant and ascites fluids were mostly around 1:20,000 and 1:1,000,000, respectively.

Characterization of Immunoglobulin Subclasses

Monoclonal antibodies produced as described above were recovered from culture fluids and characterized by Ouchterlony double diffusion tests with goat anti-mouse antibody specific to IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 and IgM. The reagents were obtained from Meloy Laboratories, Inc., Springfield, VA. Most of the clones secreted the IgG_1 subclass of immunoglobulin; very few secreted IgG_{2a}, IgG_{2b} and IgM subclasses and none secreted the IgG_3 subclass.

Characterization of SCVAP mAB-Inducing Epitope

The SCVAP epitope for the monoclonal antibodies from the thirty (30) clones selected was determined as follows. To identify the amino acid sequence(s) in SCVAP which are immunogenic epitopes to monoclonal antibodies produced against SCVAP, SCVAP was trypsin digested as described above and fragments containing residues 1-5, 6-24, 6-16 and 17-24 were obtained. When each of the monoclonal antibodies were tested against these peptide fragments by ELISA, all of them reacted with peptides containing the amino acid sequences 1-24, 6-24 and 17-24 and none reacted with those containing the 6-16 or 1-5 amino acid residues.

These data demonstrate that amino acid residues 17-24 are those which form the antigenic domain that induced production of the anti-SCVAP antibodies described above. It was surprising to find that this sequence contained the preferred epitope. This region is composed of the sequence leu - val - phe - phe - ala - glu - asp - val, in which six of the eight residues are hydrophobic and are probably restricted in a beta-helix. According to the rules that good epitopes should be hydrophilic

and mobile, one would not predict that this sequence would be especially antigenic. Because these antibodies react as strongly with purified cerebrovascular amyloid protein and amyloid core proteins as they do with SCVAP, it is concluded that this epitope is equally exposed in the natural amyloids and the synthetic peptide.

Example 2

Determination of Antigenic Determinant Commonality

In the experiments reported by this example, monoclonal antibodies purified from mouse ascites fluid were used in immunoassays in competition with homologous and heterologous antibody preparations to determine if they had epitopic sequences in common.

Preparation of Purified mABs from Ascites Fluid

From the cloned hybrid cell lines, prepared as described in Example 1, that continued to produce antibody for 3 months, ascites fluid was obtained by intraperitoneal injection of 10^7 cells into BALB/CJ mice which had been pretreated with 0.25 ml of pristane (2,6,10,14-tetramethyl-pentadecane) 1 and 8 days before injection.

Immunoglobulins of the IgG subclasses were purified from the ascites fluid with the Affi-Gel Protein A MAPS system (Bio-Rad Laboratories, Richmond, CA). The purified IgG fractions from the Protein A MAPS column were combined and dialyzed against several changes of PBS and stored at -70°C until used. -Monoclonal antibodies obtained in this manner are referred to herein as purified antibody.

Anti-SCVAP mAB - Horseradish Peroxidase (HRP) Conjugate

HRP was conjugated to anti-SCVAP monoclonal antibodies from each of the purified preparations by the periodate method described in Wilson and Nakane, In Knapp et al(eds.), Immunofluorescence and Related Techniques, Elsevier Sci. Publ. Co., Amsterdam, pp. 215-225 (1978). The conjugate solution was stored at 70°C in small aliquots after adding 1% BSA, 50% glycerol and 0.01% Thimerosal.

Identification of Antigenic Determinants

For each monoclonal antibody a fixed amount of HRP-conjugated antibody (1:5,000 dilution of stock conjugate) was mixed with various amounts (1, 10, 100 ug/ml) of either unlabelled homologous or one of the other (heterologous) monoclonal antibodies and added to ELISA plate wells coated with limiting concentrations of SCVAP antigen.

All of the monoclonal antibodies reactive to residues 17-24 (Fragment 4) effectively competed with each other and, therefore, appear to be directed either against the same epitope, or overlapping epitopes present in the amino acid sequence of residues 17-24.

Example 3

Immunocytochemistry of Alzheimer-Specific mABs

In this example, culture supernatants of 44 initial clones which exceeded 1.5 OD units in ELISA on SCVAP-coated wells were screened for immunocytochemical staining of cerebral amyloid.

Immunostaining Procedure

All supernatants were diluted with equal volumes of PBS containing 20 mg/ml BSA and applied on

6 um sections of paraffin-embedded, formalin fixed Alzheimer cortex. Bound antibodies were visualized using the avidin biotin complex technique (ABC Kit, Vector Labs, Burlingame, CA) as described in Wang et al., Acta Neuropathol.(Berl.), 62:268 (1984). Selected supernatants were also tested on sections of Alzheimer hippocampus, a non-demented aged subject and tangles-containing neuronal cell bodies isolated from Alzheimer cortex as described in Iqbal et al., Acta Neuropathol.(Berl.), 62:167 (1984). In some instances tissue sections were dephosphorylated prior to application of the antibodies using 43 ug/ml calf intestinal alkaline phosphatase type VII (Sigma Biochemicals, St. Louis, MO). In this regard, see Grundke-Iqbal et al., Proc. Nat. Acad. Sci. USA, 83:4913 (1986) and Grundke-Iqbal et al., J. Biol. Chem., 13:6084 (1986).

Immunostaining of Cerebral Amyloid - Results

Fifty (50) percent of the culture supernatants reactive with the 17-24 amino acid sequence of SCVAP also strongly reacted with amyloid present in neuritic plaques and cortical and meningeal vessels both in brain sections of Alzheimer patients and non-demented controls. No staining of Alzheimer neurofibrillary tangles or axons/neurofilaments either in tissue sections or in isolated neurons was observed with any of the supernatants.

In the case of the tangles, access of the monoclonal antibody tau-1 to microtubule associated polypeptides , tau-1 is known to be blocked by phosphorylation , as reported in Grundke-Iqbal, P.N.A.S. USA, supra. Therefore, tissue sections were also treated with alkaline phosphatase before immunostaining with the anti-SCVAP antibodies.

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Kang et al., "The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surfaceen receptor" Nature 325: 733-736 published 19 February 1989. see especially page 735.	1-23
Y	Wong et al., "Neuritio plaques and cerebrovascular amyloid in Alzheimer disease are antigenically related" Proc. Natl. Acad. Sci. USA 82: pages 8729-8732. Published December 1985. see especially page 8729.	1-23
Y	Goding, "Monoclonal antibodies: Principles and Practice" Published 1983 by Academic Press, INC. (LONDON), see pages 56-97 and pages 209-249, especially pages 230-235.	1-23

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No **
X Y	Scicutella et al., "Alz-so appears to be able to distinguish between the neuritic plaques of Alzheimer's disease and those of normal aging" soc. Neurose;Abstr. 13:1150, Published 1987. see abstract 316.5	1-8, 23 9-22
X Y	Wolozin et al., "Re-Expression of a developmentally regulated antigen in Alzheimers Disease and Down's Syndrome" Soc. Neurosci; Abstr. 13:1150 Published 1987. See Abstract 316.6	1-8, 23 9-22

However, no change in immunostaining pattern or intensity was observed when the tissue sections or tangles containing neurons were dephosphorylated prior to application of immunocytochemically reactive or unreactive antibodies.

Example 4

Immunohistology Using Alzheimer-Specific mABs

In the experiments reported by this example, purified cerebrovascular amyloid peptides and purified neuritic plaque amyloid core peptides were used for testing of recognition by monoclonal antibody specific for Fragment 4, described in Example 1.

Preparation of Cerebrovascular Amyloid Peptides

Cerebrovascular amyloid material was obtained from autopsy-derived meninges as described by Glenner and Wong, Biochem. Biophys. Res. Commun., 120:885 (1984). Cerebrovascular amyloid peptides were isolated by size exclusion chromatography and reverse-phase chromatography as follows. Cerebrovascular amyloid was dissolved in 6 M guanidine HCl-50 mM Tris HCl, pH 7.5. The solution was dialyzed and lyophilized, and the residue of amyloid peptides was dissolved in 70% formic acid, 0.1% acetonitrile. This preparation was chromatographed on a 1 x 30 cm Pharmacia HR-12 size exclusion column equilibrated with the preceding solvent. The fractions containing cerebrovascular amyloid peptide were dried and further fractionated on a J.T. Baker C-8 column 0.4 x 10 cm in a solvent containing 0-60% acetonitrile in 40% formic acid.

Preparation of Neuritic Plaque Core Peptides

Neuritic plaque amyloid cores were prepared as described by Bobin, et al., Acta. Neuropathol., 74:313 (1987). Briefly, autopsy material shown to be rich in neuritic plaque cores was homogenized in phosphate-buffered saline (PBS), pH 7.0, and centrifuged through 10 M sucrose to remove lipids. The pellet was digested with pepsin and collagenase and sedimented through Percoll. The layer containing the amyloid cores was further washed with 6 M guanidine HCl, 8 M urea, and 10% sodium deoxycholate to remove adherent lipids and cerebrovascular amyloid. Neuritic plaque amyloid core peptides were purified by the same procedure as used for the purification of the cerebrovascular amyloid peptides, except that the cores were initially dissolved in 90% formic acid.

Anti-SCVAP Fragment 4 mAB to Amyloid Core and Peptide

Monoclonal antibodies specific for amyloid residues (Fragment 4) selectively stained both plaque core amyloid and cerebrovascular amyloid in brain tissue from patients with histologically confirmed Alzheimer's disease. These monoclonal antibodies were further tested for their immunoreactivity by ELISA using 96 well plastic plates coated with the purified neuritic plaque core amyloid peptides and cerebrovascular amyloid peptides prepared as described above. All of these monoclonal antibodies reacted well with peptides from both amyloid core and cerebrovascular plaque by ELISA.

Example 5

Quantitative Diagnostic Alzheimer ELISA Method

A quantitative ELISA procedure for the detection of SCVAP in solution using the monoclonal

antibodies of the present invention was developed as follows.

Fifty (50) ul of various dilutions of monoclonal antibody SCVAP 4G8-HRP conjugate (a specific monoclonal antibody reactive to residues 17-24 of Alzheimer amyloid peptide conjugated to horseradish peroxidase) were titrated by ELISA on wells coated with the maximum amount of SCVAP antigen (5 ug/ml).

The dilution of monoclonal antibody SCVAP 4G8-HRP conjugate that gave a relative OD reading close to 1.0 when incubated with TMB for 30 minutes as described above was determined. This concentration of monoclonal antibody SCVAP 4G8-HRP conjugate was mixed with an equal volume of SCVAP solution containing varying concentrations of SCVAP and incubated overnight at 4°C. The amount of unadsorbed monoclonal antibody SCVAP 4G8-HRP conjugate was then measured by titrating the mixture on ELISA 96 well plates coated with 5 ug/ml of SCVAP. After 4 hours of incubation at room temperature the ELISA OD was determined by using TMB as the substrate.

As shown in Fig. 1, the relative OD reading between concentrations of SCVAP in this range can be quantitated using this method. The sensitivity of the method is in the range of 0.2 to 0.4 ng/100 ul of SCVAP in solution.

WHAT IS CLAIMED IS:

1. A monoclonal antibody or antigen - binding fragment thereof which is specifically reactive with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted and which does not react with other peptides of human origin.

2. The monoclonal antibody or binding fragment of claim 1 which is specifically reactive with a cerebrovascular amyloid protein whose elevated levels are characteristic of Down's syndrome and Alzheimer's disease.

3. The monoclonal antibody or binding fragment of claim 1 which is of subclass IgG_1.

4. The monoclonal antibody or binding fragment of claim 1 which is of a subclass selected from the group consisting of IgG_{2a}, IgG_{2b} and IgM.

5. The monoclonal antibody or binding fragment of claim 1 which is produced by a hybridoma formed by fusion of cells from a mouse myeloma line and antibody-producing cells from a mouse previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted and which does not react with other peptides of human origin.

6. The monoclonal antibody or binding fragment of claim 1 which is produced by a hybridoma formed by fusion of cells from a mouse myeloma line and

antibody-producing cells from a mouse previously immunized with a cerebrovascular amyloid protein whose elevated levels are characteristic of Alzheimer's disease or Down's syndrome.

7. The monoclonal antibody or binding fragment of claim 1 which is produced by a hybridoma formed by fusion of cells from a mouse NSO myeloma line and antibody-producing cells from a mouse previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted.

8. The monoclonal antibody or binding fragment of claim 1 which is produced by a hybridoma formed by fusion of cells from a mouse NSO myeloma line and antibody-producing cells from a mouse previously immunized with a cerebrovascular amyloid protein whose elevated levels are characteristic of Alzheimer's disease or Down's syndrome.

9. A monoclonal antibody which is produced by a hybridoma formed by fusion of cells from a mouse NSO myeloma line and antibody-producing cells from a mouse previously immunized with a peptide having a C-terminal sequence comprising leu - val - phe - phe - ala - glu - asp - val, or an antigen - binding fragment of said monoclonal antibody.

10. A monoclonal antibody selected from the group consisting of monoclonal antibody SCVAP 2F9 and monoclonal antibody SCVAP 4G8, or an antigen - binding fragment of said monoclonal antibody.

11. A hybridoma cell line capable of producing the monoclonal antibody of claim 1.

12. The hybridoma cell line of claim 11 which is formed by fusion of cells from a myeloma line and antibody-producing cells previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted.

13. The hybridoma cell line of claim 11 which is formed by fusion of cells from a mouse myeloma line and antibody-producing cells from a mouse previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted.

14. The hybridoma cell line of claim 11 which is formed by fusion of cells from a mouse NSO myeloma line and antibody-producing cells from a mouse previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted.

15. The hybridoma cell line of claim 11 which is formed by fusion of cells from a mouse NSO myeloma line and antibody-producing cells from a mouse previously immunized with a peptide having a C-terminal sequence comprising leu - val - phe - phe - ala - glu - asp - val.

16. A composition for quantitatively determining a peptide whose elevated levels are characteristic of Alzheimer's disease or Down's syndrome in an individual, which composition comprises the monoclonal antibody or specific - binding fragment of claim 1 and a detectable moiety which is directly or indirectly associated therewith.

17. The composition of claim 16 wherein said monoclonal antibody or specific - binding fragment is conjugated to an enzyme and said detectable moiety comprises a chromogenic redox substrate for said enzyme.

18. The composition of claim 16 wherein said monoclonal antibody or specific - binding fragment is specifically bindable with a substance attached to said detectable moiety.

19. The composition of claim 18 wherein the substance attached to said detectable moiety is an antibody specifically bindable with an immunoglobulin.

20. The composition of claim 16 wherein said monoclonal antibody is conjugated to one partner of a specific binding pair and which further comprises the other partner of said specific binding pair conjugated to said detectable moiety or a substance capable of rendering said moiety detectable.

21. The composition of claim 20 wherein one partner of said specific binding pair is selected from the group of biotin and its binding analogs and the other partner of said binding pair is selected from the group of avidin and its binding analogs.

22. The composition of either of claims 20 or 21 wherein said detectable moiety is a chromophore, fluorophore or luminophore and said substance capable of rendering said moiety detectable is an energy donor or catalyst therefor.

23. A method for quantitatively determining a peptide whose elevated levels are characteristic of Alzheimer's disease or Down's syndrome in an individual, which method comprises contacting a sample from said individual with the composition of any of claims 16-20 and quantitatively observing any detectable response.

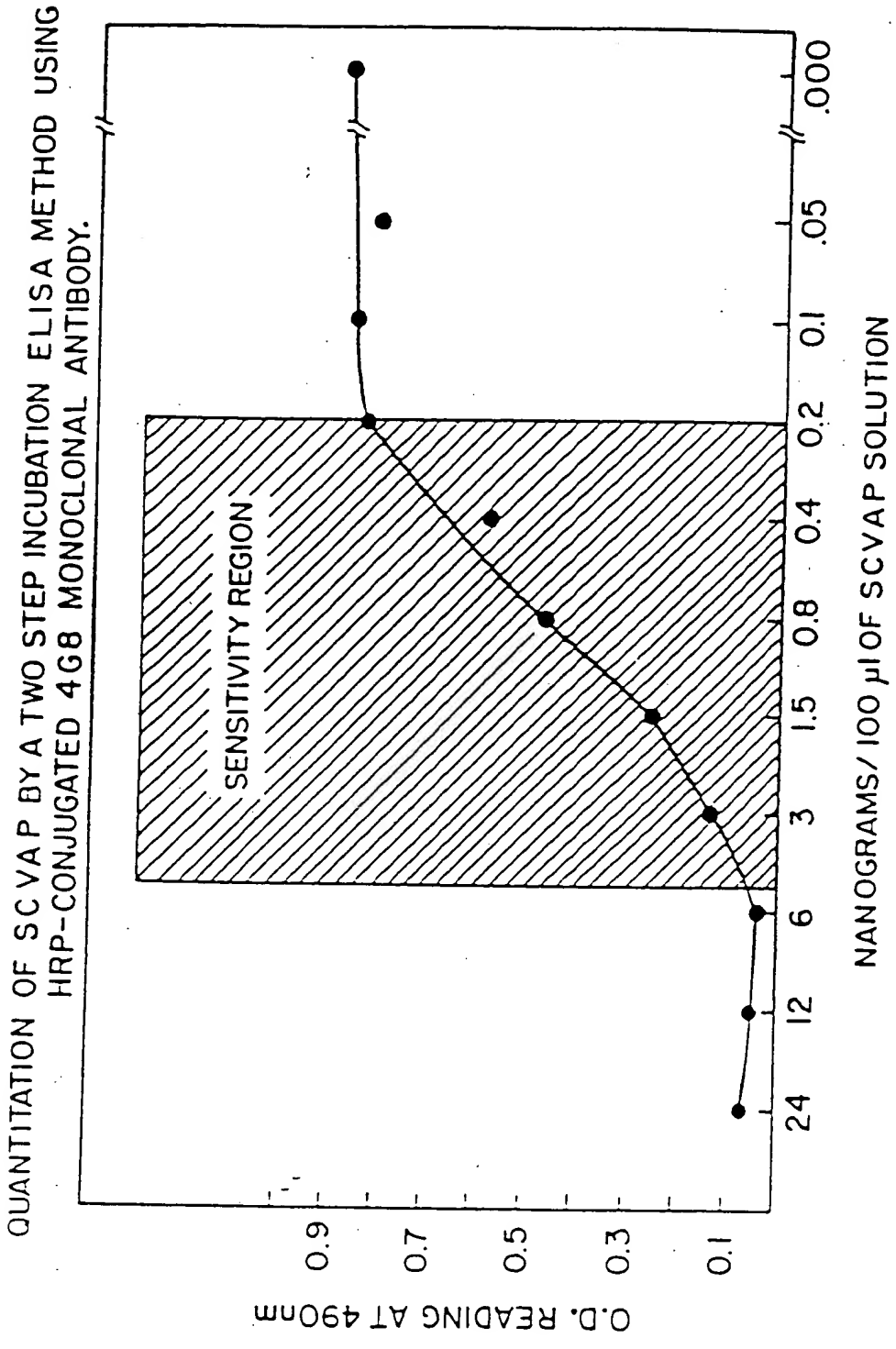

FIG. 1

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/02002

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C12N 5/20; C07K 15/28; G01N 33/533,534,535

U.S. CL: 530/387,388; 435/240.27, 188, 7.

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System ⁵	Classification Symbols
U.S.	530/387,388, 435/7, 188, 240.27; 935/104,108, 110

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁶

Databases: SIN Online (File CA, File Biosis); ALPS; For: monoclonal antibody, amylord, cerebrovascular, Alzheimers, protein, polypeptide.

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category ⁶	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X Y	US, A, 4,666,829 (GLENNER ET AL.) 19 May 1987 see the entire document.	1-8 9-23
Y	Robakis et al., "Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides", Proc. Natl. Acad. Sci 84; 4190-4194, Published June 1987. see especially p. 4192, Fig 2.	1-23
Y	Selkoe et al., "Conservation of brain amyloid proteins and humans with Alzheimer's disease" science 235: 873-877, Published 20 February 1987. see especially page 874, column 1.	1-23
Y	Glenner et al, "Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein" Biochem. Biophys. Res. Comm. 120: No. 3, 885-890. Published 16 May 1984. see especially page 889.	1-23
Y	Glenner et al, "Alzheimer's disease and Down's Syndrome: sharing of a unique cerebrovascular amyloid fibril protein" Biochem. Biophys. Res. Comm. Vol. 122 No. 3: 1131-1135. Published 16 August 1984. see especially pages 1131 and 1133, Table 1.	1-23

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ²	Date of Mailing of this International Search Report ²
25 JUNE 1990	25 JUL 1990
International Searching Authority ¹	Signature of Authorized Officer ¹⁰
ISA/US	PAULA HUTZELL

Form PCT/ISA/210 (second sheet) (May 1986)